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(71) Applicant *(for all designated States except US)*: **MERCK PATENT GMBH** [DE/DE]; Frankfurter Strasse 250, 64293 Darmstadt (DE).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **WILM, Claudia** [DE/DE]; Dahlienweg 24, 64291 Darmstadt (DE).

(74) Common Representative: **MERCK PATENT GMBH**; Frankfurter Strasse 250, 64293 Darmstadt (DE).

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(54) Title: NOVEL NATRIUM-CALCIUM EXCHANGER PROTEIN

(57) Abstract: HNCX3 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing HNCX3 polypeptides and polynucleotides in diagnostic assays.

Novel Natrium-Calcium Exchanger Protein

Field of the Invention

This invention relates to newly identified polypeptides and polynucleotides encoding such polypeptides sometimes hereinafter referred to as human Natrium(+)-Calcium(2+) exchanger form 3 (HNCX3)", to their use in diagnosis and in identifying compounds that may be agonists, antagonists that are potentially useful in therapy, and to production of such polypeptides and polynucleotides.

Background of the Invention

The drug discovery process is currently undergoing a fundamental revolution as it embraces "functional genomics", that is, high throughput genome- or gene-based biology. This approach as a means to identify genes and gene products as therapeutic targets is rapidly superceding earlier approaches based on "positional cloning". A phenotype, that is a biological function or genetic disease, would be identified and this would then be tracked back to the responsible gene, based on its genetic map position.

Functional genomics relies heavily on high-throughput DNA sequencing technologies and the various tools of bioinformatics to identify gene sequences of potential interest from the many molecular biology databases now available. There is a continuing need to identify and characterise further genes and their related polypeptides/proteins, as targets for drug discovery.

Summary of the Invention

The present invention relates to HNCX3, in particular HNCX3 polypeptides and HNCX3 polynucleotides, recombinant materials and methods for their production. Such polypeptides and polynucleotides are of interest in relation to methods of treatment of certain diseases, including,

but not limited to, acute and chronic cardiac failure of different etiologies, myocardial infarction, cardiac hypertrophy, arrhythmia, myocarditis, pulomary hypertension, cardiotoxicity (e.g induced by chemotherapy), coronary heart disease, acute and chronic renal failure, ischemic disorders of sceletal muscle, ischemic brain disorders of different ethiologies, hereinafter referred to as " diseases of the invention". In a further aspect, the invention relates to methods for identifying agonists and antagonists (*e.g.*, inhibitors) using the materials provided by the invention, and treating conditions associated with HNCX3 imbalance with the identified compounds. In a still further aspect, the invention relates to diagnostic assays for detecting diseases associated with inappropriate HNCX3 activity or levels.

Description of the Invention

In a first aspect, the present invention relates to HNCX3 polypeptides. Such polypeptides include:

(a) a polypeptide encoded by a polynucleotide comprising the sequence of SEQ ID NO:1;

(b) a polypeptide comprising a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polypeptide sequence of SEQ ID NO:2;

(c) a polypeptide comprising the polypeptide sequence of SEQ ID NO:2;

(d) a polypeptide having at least 95%, 96%, 97%, 98%, or 99% identity to the polypeptide sequence of SEQ ID NO:2;

(e) the polypeptide sequence of SEQ ID NO:2; and

(f) a polypeptide having or comprising a polypeptide sequence that has an Identity Index of 0.95, 0.96, 0.97, 0.98, or 0.99 compared to the polypeptide sequence of SEQ ID NO:2;

(g) fragments and variants of such polypeptides in (a) to (f).

Polypeptides of the present invention are believed to be members of the Na(+)-Ca(2+) exchanger family of polypeptides. They are therefore of

interest because the inhibition of Na(+)-Ca(2+) exchange might improve recovery from ischemic insults in heart, kidney and brain. The Na(+)-Ca(2+) exchanger, an ion transport protein, is expressed in the plasma membrane (PM) of virtually all animal cells. It extrudes Ca(2+) in parallel with the PM ATP-driven Ca(2+) pump. As a reversible transporter, it also mediates Ca(2+) entry in parallel with various ion channels. Five genes that code for the exchangers have been identified in mammals: three in the Na(+)-Ca(2+) exchanger family (NCX1, NCX2, and NCX3) and two in the Na(+)-Ca(2+) plus K+ family (NCKX1 and NCKX2). Alternatively spliced variants of NCX1 have been identified; dominant expression of these variants is cell type specific, which suggests that the variations are involved in targeting and/or functional differences. In cardiac myocytes, and probably other cell types, the exchanger serves a housekeeping role by maintaining a low intracellular Ca(2+) concentration. Cellular increases in Na(+) concentration lead to increases in Ca(2+) concentration mediated by the Na(+)-Ca(2+) exchanger; this is important in the therapeutic action of cardiotonic steroids like digitalis. Similarly, alterations of Na(+) and Ca(2+) apparently modulate basolateral K^+ conductance in some epithelia, signaling in some special sense organs (e.g., photoreceptors and olfactory receptors) and Ca(2+)-dependent secretion in neurons and in many secretory cells. The juxtaposition of PM and sarco(endo)plasmic reticulum membranes may permit the PM Na(+)-Ca(2+) exchanger to regulate sarco(endo)plasmic reticulum Ca(2+) stores and influence cellular Ca(2+) signaling (Blaustein and Lederer (1999) Physiol Rev. 79(3):763-854). The human NCX1 gene is located on chromosome 2p22-p23 (Shieh, et al. (1992) Genomics 12(3):616-617); Kraev, et al. (1996) Genomics 37(1):105-112; McDaniel, et al. (1993) Cytogenet Cell Genet. 63(3):192-193). NCX1 is expressed most abundantly in the heart and next in the brain (Komuro, et al. (1992) Proc Natl Acad Sci U S A 89(10):4769-4773). The gene of the hereby described human HNCX3 is located on chromosome 14 and is expressed in human brain. Among the known Na(+)-Ca(2+) exchanger genes of rat, cat, and the human NCX1, the human HNCX3 exhibits the highest degree of homology to rat NCX3.

In the failing human heart proteins involved in calcium removal were significantly altered. Sarcoplasmic reticulum (SR)-Ca(2+)-ATPase levels and the ratio of SR-Ca(2+)-ATPase to its inhibitory protein

phospholamban were significantly decreased, and Na(+)-Ca(2+) exchanger levels and the ratio of Na(+)-Ca(2+) exchanger to SR-Ca(2+)-ATPase were significantly increased. SR-Ca(2+)-ATPase levels were closely correlated to systolic function as evaluated by frequency potentiation of contractile force. The frequency-dependent rise of diastolic force was inversely correlated with protein levels of Na(+)-Ca(2+) exchanger. These findings indicate that altered expression of SR-Ca(2+)-ATPase and Na(+)-Ca(2+) exchanger is relevant for altered systolic and diastolic function in human heart failure (Lehnart, et al. (1998) Ann N Y Acad Sci. 853:220-230).

In ischemic acute renal failure (ARF) in rats pretreatment with a Na(+)-Ca(2+) exchange inhibitor, markedly attenuated the ARF-induced renal dysfunction. Histopathological examination of the kidney of ARF rats revealed severe renal damage, which was suppressed by the Na(+)-Ca(2+) exchange inhibitor. Activation of the reverse mode of Na(+)-Ca(2+) exchange seems to play an important role in the pathogenesis of ARF (Kuro, et al. (1999) Jpn J Pharmacol. 81(2):247-251).

Intracellular pH may be an important variable regulating neurotransmitter release. A number of pathological conditions, such as anoxia and ischemia, are known to influence intracellular pH, causing acidification of brain cells and excitotoxicity. Excessive release of glutamate could be implicated in excitotoxic insults after anoxic or ischemic episodes. During recovery from intracellular acidification a massive activation of neurotransmitter release occurs in hippocampal neurons because of the successive activation of the Na(+)-H(+) and Na(+)-Ca(2+) exchangers in nerve terminals that leads to an elevation of intracellular calcium. The rise in free Ca(2+) was blocked and the recovery and the recovery of hippocampal neurons was improved by a Na(+)-Ca(2+) exchange inhibitor (Trudeau, et al. (1999) J Neurophysiol. 81(6):2627-2635; Schroder, et al. (1999) Neuropharmacology 38(2):319-321).

The existence of multiple Na(+)-Ca(2+) exchanger isoforms may provide flexibility for regulation and expression. Tissue selectivity or selective expression of isoforms in certain pathological conditions may allow more specific pharmacological approaches.

The biological properties of the HNCX3 are hereinafter referred to as "biological activity of HNCX3" or "HNCX3 activity". Preferably, a polypeptide of the present invention exhibits at least one biological activity of HNCX3.

Polypeptides of the present invention also includes variants of the aforementioned polypeptides, including all allelic forms and splice variants. Such polypeptides vary from the reference polypeptide by insertions, deletions, and substitutions that may be conservative or non-conservative, or any combination thereof. Particularly preferred variants are those in which several, for instance from 50 to 30, from 30 to 20, from 20 to 10, from 10 to 5, from 5 to 3, from 3 to 2, from 2 to 1 or 1 amino acids are inserted, substituted, or deleted, in any combination.

Preferred fragments of polypeptides of the present invention include a polypeptide comprising an amino acid sequence having at least 30, 50 or 100 contiguous amino acids from the amino acid sequence of SEQ ID NO: 2, or a polypeptide comprising an amino acid sequence having at least 30, 50 or 100 contiguous amino acids truncated or deleted from the amino acid sequence of SEQ ID NO: 2. Preferred fragments are biologically active fragments that mediate the biological activity of HNCX3, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also preferred are those fragments that are antigenic or immunogenic in an animal, especially in a human.

Fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these variants may be employed as intermediates for producing the full-length polypeptides of the invention.The polypeptides of the present invention may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence that contains secretory or leader sequences, pro-sequences, sequences that aid in purification, for instance multiple histidine residues, or an additional sequence for stability during recombinant production.

Polypeptides of the present invention can be prepared in any suitable manner, for instance by isolation form naturally occuring sources, from genetically engineered host cells comprising expression systems (*vide*

infra) or by chemical synthesis, using for instance automated peptide synthesisers, or a combination of such methods.. Means for preparing such polypeptides are well understood in the art.

In a further aspect, the present invention relates to HNCX3 polynucleotides. Such polynucleotides include:

(a) a polynucleotide comprising a polynucleotide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polynucleotide squence of SEQ ID NO:1;

(b) a polynucleotide comprising the polynucleotide of SEQ ID NO:1;

(c) a polynucleotide having at least 95%, 96%, 97%, 98%, or 99% identity to the polynucleotide of SEQ ID NO:1;

(d) the polynucleotide of SEQ ID NO:1;

(e) a polynucleotide comprising a polynucleotide sequence encoding a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polypeptide sequence of SEQ ID NO:2;

(f) a polynucleotide comprising a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2;

(g) a polynucleotide having a polynucleotide sequence encoding a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polypeptide sequence of SEQ ID NO:2;

(h) a polynucleotide encoding the polypeptide of SEQ ID NO:2;

(i) a polynucleotide having or comprising a polynucleotide sequence that has an Identity Index of 0.95, 0.96, 0.97, 0.98, or 0.99 compared to the polynucleotide sequence of SEQ ID NO:1;

(j) a polynucleotide having or comprising a polynucleotide sequence encoding a polypeptide sequence that has an Identity Index of 0.95, 0.96, 0.97, 0.98, or 0.99 compared to the polypeptide sequence of SEQ ID NO:2; and

polynucleotides that are fragments and variants of the above mentioned polynucleotides or that are complementary to above mentioned polynucleotides, over the entire length thereof.

Preferred fragments of polynucleotides of the present invention include a polynucleotide comprising an nucleotide sequence having at least 15, 30, 50 or 100 contiguous nucleotides from the sequence of SEQ ID NO: 1, or a polynucleotide comprising an sequence having at least 30, 50 or 100 contiguous nucleotides truncated or deleted from the sequence of SEQ ID NO: 1.

Preferred variants of polynucleotides of the present invention include splice variants, allelic variants, and polymorphisms, including polynucleotides having one or more single nucleotide polymorphisms (SNPs).

Polynucleotides of the present invention also include polynucleotides encoding polypeptide variants that comprise the amino acid sequence of SEQ ID NO:2 and in which several, for instance from 50 to 30, from 30 to 20, from 20 to 10, from 10 to 5, from 5 to 3, from 3 to 2, from 2 to 1 or 1 amino acid residues are substituted, deleted or added, in any combination.

In a further aspect, the present invention provides polynucleotides that are RNA transcripts of the DNA sequences of the present invention. Accordingly, there is provided an RNA polynucleotide that:

(a) comprises an RNA transcript of the DNA sequence encoding the polypeptide of SEQ ID NO:2;

(b) is the RNA transcript of the DNA sequence encoding the polypeptide of SEQ ID NO:2;

(c) comprises an RNA transcript of the DNA sequence of SEQ ID NO:1; or

(d) is the RNA transcript of the DNA sequence of SEQ ID NO:1;

and RNA polynucleotides that are complementary thereto.

The polynucleotide sequence of SEQ ID NO:1 shows homology with rat sodium-calcium exchanger form 3 (U53420; GenBANK; Nicoll et al., J. Biol. Chem. (1996) 271:24914-24921). The polynucleotide sequence of SEQ ID NO:1 is a cDNA sequence that encodes the polypeptide of SEQ ID NO:2. The polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence of SEQ ID NO:1 or it may be a sequence other than SEQ ID NO:1, which, as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2. The polypeptide of the SEQ ID NO:2 is related to other proteins of the Na(+)-Ca(2+) exchanger family, having homology and/or structural similarity with rat sodium-calcium exchanger form 3 (P70549; Swiss-Prot; Nicoll et al., J. Biol. Chem. (1996) 271:24914-24921).

Preferred polypeptides and polynucleotides of the present invention are expected to have, *inter alia*, similar biological functions/properties to their homologous polypeptides and polynucleotides. Furthermore, preferred polypeptides and polynucleotides of the present invention have at least one HNCX3 activity.

Polynucleotides of the present invention may be obtained using standard cloning and screening techniques from a cDNA library derived from mRNA in cells of human fetal and adult brain, retina, sceletal muscle, and kidney (see for instance, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

When polynucleotides of the present invention are used for the recombinant production of polypeptides of the present invention, the polynucleotide may include the coding sequence for the mature polypeptide, by itself, or the coding sequence for the mature polypeptide in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain

preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, Proc Natl Acad Sci USA (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Polynucleotides that are identical, or have sufficient identity to a polynucleotide sequence of SEQ ID NO:1, may be used as hybridization probes for cDNA and genomic DNA or as primers for a nucleic acid amplification reaction (for instance, PCR). Such probes and primers may be used to isolate full-length cDNAs and genomic clones encoding polypeptides of the present invention and to isolate cDNA and genomic clones of other genes (including genes encoding paralogs from human sources and orthologs and paralogs from species other than human) that have a high sequence similarity to SEQ ID NO:1, typically at least 95% identity. Preferred probes and primers will generally comprise at least 15 nucleotides, preferably, at least 30 nucleotides and may have at least 50, if not at least 100 nucleotides. Particularly preferred probes will have between 30 and 50 nucleotides. Particularly preferred primers will have between 20 and 25 nucleotides.

A polynucleotide encoding a polypeptide of the present invention, including homologs from species other than human, may be obtained by a process comprising the steps of screening a library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO: 1 or a fragment thereof, preferably of at least 15 nucleotides; and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to the skilled artisan. Preferred stringent hybridization conditions include overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA; followed by washing the filters in 0.1x SSC at about 65°C. Thus the present invention also includes isolated polynucleotides, preferably with a nucleotide sequence of at least 100, obtained by screening a library under stringent hybridization conditions with a labeled

probe having the sequence of SEQ ID NO:1 or a fragment thereof, preferably of at least 15 nucleotides.

The skilled artisan will appreciate that, in many cases, an isolated cDNA sequence will be incomplete, in that the region coding for the polypeptide does not extend all the way through to the 5' terminus. This is a consequence of reverse transcriptase, an enzyme with inherently low "processivity" (a measure of the ability of the enzyme to remain attached to the template during the polymerisation reaction), failing to complete a DNA copy of the mRNA template during first strand cDNA synthesis.

There are several methods available and well known to those skilled in the art to obtain full-length cDNAs, or extend short cDNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman et al., Proc Nat Acad Sci USA 85, 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon (trade mark) technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon (trade mark) technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the cDNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using 'nested' primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the known gene sequence). The products of this reaction can then be analysed by DNA sequencing and a full-length cDNA constructed either by joining the product directly to the existing cDNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

Recombinant polypeptides of the present invention may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems comprising a

polynucleotide or polynucleotides of the present invention, to host cells which are genetically engineered with such expression sytems and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Polynucleotides may be introduced into host cells by methods described in many standard laboratory manuals, such as Davis et al., Basic Methods in Molecular Biology (1986) and Sambrook *et al.*(*ibid*). Preferred methods of introducing polynucleotides into host cells include, for instance, calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as *Streptococci*, *Staphylococci*, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used, for instance, chromosomal, episomal and virus-derived systems, *e.g.*, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector that is able to maintain, propagate or express a polynucleotide to produce a polypeptide in a host may be used. The appropriate polynucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as,

for example, those set forth in Sambrook *et al.*, (*ibid*). Appropriate secretion signals may be incorporated into the desired polypeptide to allow secretion of the translated protein into the lumen of the endoplasmic reticulum, the periplasmic space or the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If a polypeptide of the present invention is to be expressed for use in screening assays, it is generally preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If the polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide. If produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and/or purification.

Polynucleotides of the present invention may be used as diagnostic reagents, through detecting mutations in the associated gene. Detection of a mutated form of the gene characterised by the polynucleotide of SEQ ID NO:1 in the cDNA or genomic sequence and which is associated with a dysfunction will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, or susceptibility to a disease, which results from under-expression, over-expression or altered spatial or temporal expression of the gene. Individuals carrying mutations in the gene may be detected at the DNA level by a variety of techniques well known in the art.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or it may be amplified enzymatically by using PCR, preferably RT-PCR, or other amplification techniques prior to

analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled HNCX3 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence difference may also be detected by alterations in the electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing (see, for instance, Myers *et al.*, Science (1985) 230:1242). Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (see Cotton *et al.*, Proc Natl Acad Sci USA (1985) 85: 4397-4401).

An array of oligonucleotides probes comprising HNCX3 polynucleotide sequence or fragments thereof can be constructed to conduct efficient screening of *e.g.*, genetic mutations. Such arrays are preferably high density arrays or grids. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability, see, for example, M.Chee et al., Science, 274, 610-613 (1996) and other references cited therein.

Detection of abnormally decreased or increased levels of polypeptide or mRNA expression may also be used for diagnosing or determining susceptibility of a subject to a disease of the invention. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, nucleic acid amplification, for instance PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as a polypeptide of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagonostic kit comprising:

(a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment or an RNA transcript thereof;

(b) a nucleotide sequence complementary to that of (a);

(c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO:2 or a fragment thereof; or

(d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID NO:2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or susceptibility to a disease, particularly diseases of the invention, amongst others.

The polynucleotide sequences of the present invention are valuable for chromosome localisation studies. The sequence is specifically targeted to, and can hybridize with, a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, for example, V. McKusick, Mendelian Inheritance in Man (available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (co-inheritance of physically adjacent genes). Precise human chromosomal localisations for a genomic sequence (gene fragment etc.) can be determined using Radiation Hybrid (RH) Mapping (Walter, M. Spillett, D., Thomas, P., Weissenbach, J., and Goodfellow, P., (1994) A method for constructing radiation hybrid maps of whole genomes, Nature Genetics 7, 22-28). A number of RH panels are available from Research Genetics (Huntsville, AL, USA) e.g. the GeneBridge4 RH panel (Hum Mol Genet 1996 Mar;5(3):339-46 A radiation hybrid map of the human genome. Gyapay G, Schmitt K, Fizames C, Jones H, Vega-Czarny N, Spillett D, Muselet D, Prud'Homme

JF, Dib C, Auffray C, Morissette J, Weissenbach J, Goodfellow PN). To determine the chromosomal location of a gene using this panel, 93 PCRs are performed using primers designed from the gene of interest on RH DNAs. Each of these DNAs contains random human genomic fragments maintained in a hamster background (human / hamster hybrid cell lines). These PCRs result in 93 scores indicating the presence or absence of the PCR product of the gene of interest. These scores are compared with scores created using PCR products from genomic sequences of known location. This comparison is conducted at http://www.genome.wi.mit.edu/. The gene of the present invention maps to human chromosome 14.

The polynucleotide sequences of the present invention are also valuable tools for tissue expression studies. Such studies allow the determination of expression patterns of polynucleotides of the present invention which may give an indication as to the expression patterns of the encoded polypeptides in tissues, by detecting the mRNAs that encode them. The techniques used are well known in the art and include in situ hydridisation techniques to clones arrayed on a grid, such as cDNA microarray hybridisation (Schena *et al*, Science, 270, 467-470, 1995 and Shalon *et al*, Genome Res, 6, 639-645, 1996) and nucleotide amplification techniques such as PCR. A preferred method uses the TAQMAN (Trade mark) technology available from Perkin Elmer. Results from these studies can provide an indication of the normal function of the polypeptide in the organism. In addition, comparative studies of the normal expression pattern of mRNAs with that of mRNAs encoded by an alternative form of the same gene (for example, one having an alteration in polypeptide coding potential or a regulatory mutation) can provide valuable insights into the role of the polypeptides of the present invention, or that of inappropriate expression thereof in disease. Such inappropriate expression may be of a temporal, spatial or simply quantitative nature.

The polypeptides of the present invention are expressed in fetal and adult brain, and retina.

A further aspect of the present invention relates to antibodies. The polypeptides of the invention or their fragments, or cells expressing them, can be used as immunogens to produce antibodies that are immunospecific for polypeptides of the present invention. The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against polypeptides of the present invention may be obtained by administering the polypeptides or epitope-bearing fragments, or cells to an animal, preferably a non-human animal, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., Nature (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, Immunology Today (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies, such as those described in U.S. Patent No. 4,946,778, can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms, including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography. Antibodies against polypeptides of the present invention may also be employed to treat diseases of the invention, amongst others.

Polypeptides and polynucleotides of the present invention may also be used as vaccines. Accordingly, in a further aspect, the present invention relates to a method for inducing an immunological response in a mammal that comprises inoculating the mammal with a polypeptide of the present invention, adequate to produce antibody and/or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said animal from disease, whether that disease is already established within the individual or not. An immunological response in a

mammal may also be induced by a method comprises delivering a polypeptide of the present invention *via* a vector directing expression of the polynucleotide and coding for the polypeptide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases of the invention. One way of administering the vector is by accelerating it into the desired cells as a coating on particles or otherwise. Such nucleic acid vector may comprise DNA, RNA, a modified nucleic acid, or a DNA/RNA hybrid. For use a vaccine, a polypeptide or a nucleic acid vector will be normally provided as a vaccine formulation (composition). The formulation may further comprise a suitable carrier. Since a polypeptide may be broken down in the stomach, it is preferably administered parenterally (for instance, subcutaneous, intramuscular, intravenous, or intradermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions that may contain anti-oxidants, buffers, bacteriostats and solutes that render the formulation instonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions that may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Polypeptides of the present invention have one or more biological functions that are of relevance in one or more disease states, in particular the diseases of the invention hereinbefore mentioned. It is therefore useful to to identify compounds that stimulate or inhibit the function or level of the polypeptide. Accordingly, in a further aspect, the present invention provides for a method of screening compounds to identify those that stimulate or inhibit the function or level of the polypeptide. Such methods identify agonists or antagonists that may be employed for therapeutic and prophylactic purposes for such diseases of the invention as hereinbefore mentioned. Compounds may be identified from a variety of sources, for

example, cells, cell-free preparations, chemical libraries, collections of chemical compounds, and natural product mixtures. Such agonists or antagonists so-identified may be natural or modified substrates, ligands, receptors, enzymes, etc., as the case may be, of the polypeptide; a structural or functional mimetic thereof (see Coligan *et al.*, Current Protocols in Immunology 1(2):Chapter 5 (1991)) or a small molecule.

The screening method may simply measure the binding of a candidate compound to the polypeptide, or to cells or membranes bearing the polypeptide, or a fusion protein thereof, by means of a label directly or indirectly associated with the candidate compound. Alternatively, the screening method may involve measuring or detecting (qualitatively or quantitatively) the competitive binding of a candidate compound to the polypeptide against a labeled competitor (*e.g.* agonist or antagonist). Further, these screening methods may test whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the cells bearing the polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Further, the screening methods may simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide of the present invention, to form a mixture, measuring a HNCX3 activity in the mixture, and comparing the HNCX3 activity of the mixture to a control mixture which contains no candidate compound.

Polypeptides of the present invention may be employed in conventional low capacity screening methods and also in high-throughput screening (HTS) formats. Such HTS formats include not only the well-established use of 96- and, more recently, 384-well micotiter plates but also emerging methods such as the nanowell method described by Schullek et al, Anal Biochem., 246, 20-29, (1997).

Fusion proteins, such as those made from Fc portion and HNCX3 polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists for the polypeptide of the present invention (see D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995)).

Screening techniques

The polynucleotides, polypeptides and antibodies to the polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents that may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

A polypeptide of the present invention may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. These include, but are not limited to, ligand binding and crosslinking assays in which the polypeptide is labeled with a radioactive isotope (for instance, ^{125}I), chemically modified (for instance, biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (cells, cell membranes, cell supernatants, tissue extracts, bodily fluids). Other methods include biophysical techniques such as surface plasmon resonance and spectroscopy. These screening methods may also be used to identify agonists and antagonists of the polypeptide that compete with the binding of the polypeptide to its receptors, if any. Standard methods for conducting such assays are well understood in the art.

Examples of antagonists of polypeptides of the present invention include antibodies or, in some cases, oligonucleotides or proteins that are closely related to the ligands, substrates, receptors, enzymes, etc., as the case may be, of the polypeptide, *e.g.*, a fragment of the ligands, substrates, receptors, enzymes, etc.; or a small molecule that bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Screening methods may also involve the use of transgenic technology and HNCX3 gene. The art of constructing transgenic animals is well

established. For example, the HNCX3 gene may be introduced through microinjection into the male pronucleus of fertilized oocytes, retroviral transfer into pre- or post-implantation embryos, or injection of genetically modified, such as by electroporation, embryonic stem cells into host blastocysts. Particularly useful transgenic animals are so-called "knock-in" animals in which an animal gene is replaced by the human equivalent within the genome of that animal. Knock-in transgenic animals are useful in the drug discovery process, for target validation, where the compound is specific for the human target. Other useful transgenic animals are so-called "knock-out" animals in which the expression of the animal ortholog of a polypeptide of the present invention and encoded by an endogenous DNA sequence in a cell is partially or completely annulled. The gene knock-out may be targeted to specific cells or tissues, may occur only in certain cells or tissues as a consequence of the limitations of the technology, or may occur in all, or substantially all, cells in the animal. Transgenic animal technology also offers a whole animal expression-cloning system in which introduced genes are expressed to give large amounts of polypeptides of the present invention

Screening kits for use in the above described methods form a further aspect of the present invention. Such screening kits comprise:

(a) a polypeptide of the present invention;

(b) a recombinant cell expressing a polypeptide of the present invention;

(c) a cell membrane expressing a polypeptide of the present invention; or

(d) an antibody to a polypeptide of the present invention;

which polypeptide is preferably that of SEQ ID NO:2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Glossary

The following definitions are provided to facilitate understanding of certain terms used frequently hereinbefore.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an

Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from its natural state, *i.e.*, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Moreover, a polynucleotide or polypeptide that is introduced into an organism by transformation, genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said organism, which organism may be living or non-living.

"Polynucleotide" generally refers to any polyribonucleotide (RNA) or polydeoxribonucleotide (DNA), which may be unmodified or modified RNA or DNA. "Polynucleotides" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term "polynucleotide" also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any polypeptide comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds,

i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques that are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications may occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present to the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from post-translation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, biotinylation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination (see, for instance, Proteins - Structure and Molecular Properties, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993; Wold, F., Post-translational Protein Modifications: Perspectives and Prospects, 1-12, in Post-translational Covalent Modification of Proteins, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", Meth Enzymol, 182, 626-646, 1990, and Rattan *et al.*, "Protein Synthesis: Post-translational Modifications and Aging", Ann NY Acad Sci, 663, 48-62, 1992).

"Fragment" of a polypeptide sequence refers to a polypeptide sequence that is shorter than the reference sequence but that retains essentially the same biological function or activity as the reference polypeptide. "Fragment" of a polynucleotide sequence refers to a polynucloetide sequence that is shorter than the reference sequence of SEQ ID NO:1..

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains the essential properties thereof. A typical variant of a polynucleotide differs in nucleotide sequence from the reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from the reference polypeptide. Generally, alterations are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, insertions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. Typical conservative substitutions include Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe and Tyr. A variant of a polynucleotide or polypeptide may be naturally occurring such as an allele, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis. Also included as variants are polypeptides having one or more post-translational modifications, for instance glycosylation, phosphorylation, methylation, ADP ribosylation and the like. Embodiments include methylation of the N-terminal amino acid, phosphorylations of serines and threonines and modification of C-terminal glycines.

"Allele" refers to one of two or more alternative forms of a gene occuring at a given locus in the genome.

"Polymorphism" refers to a variation in nucleotide sequence (and encoded polypeptide sequence, if relevant) at a given position in the genome within a population.

"Single Nucleotide Polymorphism" (SNP) refers to the occurence of nucleotide variability at a single nucleotide position in the genome, within a population. An SNP may occur within a gene or within intergenic regions of the genome. SNPs can be assayed using Allele Specific Amplification (ASA). For the process at least 3 primers are required. A common primer is used in reverse complement to the polymorphism being assayed. This common primer can be between 50 and 1500 bps from the polymorphic base. The other two (or more) primers are identical to each other except that the final 3' base wobbles to match one of the two (or more) alleles that make up the polymorphism. Two (or more) PCR reactions are then conducted on sample DNA, each using the common primer and one of the Allele Specific Primers.

"Splice Variant" as used herein refers to cDNA molecules produced from RNA molecules initially transcribed from the same genomic DNA sequence but which have undergone alternative RNA splicing. Alternative RNA splicing occurs when a primary RNA transcript undergoes splicing, generally for the removal of introns, which results in the production of more than one mRNA molecule each of that may encode different amino acid sequences. The term splice variant also refers to the proteins encoded by the above cDNA molecules.

"Identity" reflects a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, determined by comparing the sequences. In general, identity refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of the two polynucleotide or two polypeptide sequences, respectively, over the length of the sequences being compared.

"% Identity" - For sequences where there is not an exact correspondence, a "% identity" may be determined. In general, the two sequences to be compared are aligned to give a maximum correlation between the sequences. This may include inserting "gaps" in either one or both sequences, to enhance the degree of alignment. A % identity may be determined over the whole length of each of the sequences being

compared (so-called global alignment), that is particularly suitable for sequences of the same or very similar length, or over shorter, defined lengths (so-called local alignment), that is more suitable for sequences of unequal length.

"Similarity" is a further, more sophisticated measure of the relationship between two polypeptide sequences. In general, "similarity" means a comparison between the amino acids of two polypeptide chains, on a residue by residue basis, taking into account not only exact correspondences between a between pairs of residues, one from each of the sequences being compared (as for identity) but also, where there is not an exact correspondence, whether, on an evolutionary basis, one residue is a likely substitute for the other. This likelihood has an associated "score" from which the "% similarity" of the two sequences can then be determined.

Methods for comparing the identity and similarity of two or more sequences are well known in the art. Thus for instance, programs available in the Wisconsin Sequence Analysis Package, version 9.1 (Devereux J et al, Nucleic Acids Res, 12, 387-395, 1984, available from Genetics Computer Group, Madison, Wisconsin, USA), for example the programs BESTFIT and GAP, may be used to determine the % identity between two polynucleotides and the % identity and the % similarity between two polypeptide sequences. BESTFIT uses the "local homology" algorithm of Smith and Waterman (J Mol Biol, 147,195-197, 1981, Advances in Applied Mathematics, 2, 482-489, 1981) and finds the best single region of similarity between two sequences. BESTFIT is more suited to comparing two polynucleotide or two polypeptide sequences that are dissimilar in length, the program assuming that the shorter sequence represents a portion of the longer. In comparison, GAP aligns two sequences, finding a "maximum similarity", according to the algorithm of Neddleman and Wunsch (J Mol Biol, 48, 443-453, 1970). GAP is more suited to comparing sequences that are approximately the same length and an alignment is expected over the entire length. Preferably, the parameters "Gap Weight" and "Length Weight" used in each program are 50 and 3, for polynucleotide sequences and 12 and 4 for polypeptide sequences, respectively. Preferably, % identities and similarities are determined when the two sequences being compared are optimally aligned.

Other programs for determining identity and/or similarity between sequences are also known in the art, for instance the BLAST family of programs (Altschul S F et al, J Mol Biol, 215, 403-410, 1990, Altschul S F et al, Nucleic Acids Res., 25:389-3402, 1997, available from the National Center for Biotechnology Information (NCBI), Bethesda, Maryland, USA and accessible through the home page of the NCBI at www.ncbi.nlm.nih.gov) and FASTA (Pearson W R, Methods in Enzymology, 183, 63-99, 1990; Pearson W R and Lipman D J, Proc Nat Acad Sci USA, 85, 2444-2448,1988, available as part of the Wisconsin Sequence Analysis Package).

Preferably, the BLOSUM62 amino acid substitution matrix (Henikoff S and Henikoff J G, Proc. Nat. Acad Sci. USA, 89, 10915-10919, 1992) is used in polypeptide sequence comparisons including where nucleotide sequences are first translated into amino acid sequences before comparison.

Preferably, the program BESTFIT is used to determine the % identity of a query polynucleotide or a polypeptide sequence with respect to a reference polynucleotide or a polypeptide sequence, the query and the reference sequence being optimally aligned and the parameters of the program set at the default value, as hereinbefore described.

"Identity Index" is a measure of sequence relatedness which may be used to compare a candidate sequence (polynucleotide or polypeptide) and a reference sequence. Thus, for instance, a candidate polynucleotide sequence having, for example, an Identity Index of 0.95 compared to a reference polynucleotide sequence is identical to the reference sequence except that the candidate polynucleotide sequence may include on average up to five differences per each 100 nucleotides of the reference sequence. Such differences are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion. These differences may occur at the 5' or 3' terminal positions of the reference polynucleotide sequence or anywhere between these terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. In other words, to obtain a polynucleotide sequence having an Identity Index of 0.95 compared to a reference polynucleotide sequence, an average of up to 5

in every 100 of the nucleotides of the in the reference sequence may be deleted, substituted or inserted, or any combination thereof, as hereinbefore described. The same applies *mutatis mutandis* for other values of the Identity Index, for instance 0.96, 0.97, 0.98 and 0.99.

Similarly, for a polypeptide, a candidate polypeptide sequence having, for example, an Identity Index of 0.95 compared to a reference polypeptide sequence is identical to the reference sequence except that the polypeptide sequence may include an average of up to five differences per each 100 amino acids of the reference sequence. Such differences are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion. These differences may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between these terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. In other words, to obtain a polypeptide sequence having an Identity Index of 0.95 compared to a reference polypeptide sequence, an average of up to 5 in every 100 of the amino acids in the reference sequence may be deleted, substituted or inserted, or any combination thereof, as hereinbefore described. The same applies *mutatis mutandis* for other values of the Identity Index, for instance 0.96, 0.97, 0.98 and 0.99.

The relationship between the number of nucleotide or amino acid differences and the Identity Index may be expressed in the following equation:

$$n_a \leq x_a - (x_a \bullet I),$$

in which:

n_a is the number of nucleotide or amino acid differences,

x_a is the total number of nucleotides or amino acids in SEQ ID NO:1 or SEQ ID NO:2, respectively,

I is the Identity Index ,

$\bullet$ is the symbol for the multiplication operator, and

in which any non-integer product of x_a and I is rounded down to the nearest integer prior to subtracting it from x_a.

"Homolog" is a generic term used in the art to indicate a polynucleotide or polypeptide sequence possessing a high degree of sequence relatedness to a reference sequence. Such relatedness may be quantified by determining the degree of identity and/or similarity between the two sequences as hereinbefore defined. Falling within this generic term are the terms "ortholog", and "paralog". "Ortholog" refers to a polynucleotide or polypeptide that is the functional equivalent of the polynucleotide or polypeptide in another species. "Paralog" refers to a polynucleotideor polypeptide that within the same species which is functionally similar.

"Fusion protein" refers to a protein encoded by two, unrelated, fused genes or fragments thereof. Examples have been disclosed in US 5541087, 5726044. In the case of Fc-HNCX3, employing an immunoglobulin Fc region as a part of a fusion protein is advantageous for performing the functional expression of Fc-HNCX3 or fragments of HNCX3, to improve pharmacokinetic properties of such a fusion protein when used for therapy and to generate a dimeric HNCX3. The Fc-HNCX3 DNA construct comprises in 5' to 3' direction, a secretion cassette, i.e. a signal sequence that triggers export from a mammalian cell, DNA encoding an immunoglobulin Fc region fragment, as a fusion partner, and a DNA encoding HNCX3 or fragments thereof. In some uses it would be desirable to be able to alter the intrinsic functional properties (complement binding, Fc-Receptor binding) by mutating the functional Fc sides while leaving the rest of the fusion protein untouched or delete the Fc part completely after expression.

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

Claims

1. A polypeptide selected from the group consisting of:

(a) a polypeptide encoded by a polynucleotide comprising the sequence of SEQ ID NO:1;

(b) a polypeptide comprising a polypeptide sequence having at least 95% identity to the polypeptide sequence of SEQ ID NO:2;

c) a polypeptide having at least 95% identity to the polypeptide sequence of SEQ ID NO:2;

d) the polypeptide sequence of SEQ ID NO:2 and

(e) fragments and variants of such polypeptides in (a) to (d).

2. The polypeptide of claim 1 comprising the polypeptide sequence of SEQ ID NO:2.

3. The polypeptide of claim 1 which is the polypeptide sequence of SEQ ID NO:2.

4. A polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising a polynucleotide sequence having at least 95% identity to the polynucleotide sequence of SEQ ID NO:1;

(b) a polynucleotide having at least 95% identity to the polynucleotide of SEQ ID NO:1;

(c) a polynucleotide comprising a polynucleotide sequence encoding a polypeptide sequence having at least 95% identity to the polypeptide sequence of SEQ ID NO:2;

(d) a polynucleotide having a polynucleotide sequence encoding a polypeptide sequence having at least 95% identity to the polypeptide sequence of SEQ ID NO:2;

(e) a polynucleotide with a nucleotide sequence of at least 100 nucleotides obtained by screening a library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO: 1 or a fragment thereof having at least 15 nucleotides;

(f) a polynucleotide which is the RNA equivalent of a polynucleotide of (a) to (e);

(g) a polynucleotide sequence complementary to said polynucleotide of any one of (a) to (f), and

(h) polynucleotides that are variants or fragments of the polynucleotides of any one of (a) to (g) or that are complementary to above mentioned polynucleotides, over the entire length thereof.

5. A polynucleotide of claim 4 selected from the group consisting of:

(a) a polynucleotide comprising the polynucleotide of SEQ ID NO:1;

(b) the polynucleotide of SEQ ID NO:1;

(c) a polynucleotide comprising a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2; and

(d) a polynucleotide encoding the polypeptide of SEQ ID NO:2.

6. An expression system comprising a polynucleotide capable of producing a polypeptide of any one of claim 1-3 when said expression vector is present in a compatible host cell.

7. A recombinant host cell comprising the expression vector of claim 6 or a membrane thereof expressing the polypeptide of any one of claim 1-3.

8. A process for producing a polypeptide of any one of claim 1-3 comprising the step of culturing a host cell as defined in claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

9. A fusion protein consisting of the Immunoglobulin Fc-region and a polypeptide any one one of claims 1-3.

10. An antibody immunospecific for the polypeptide of any one of claims 1 to 3.

11. A method for screening to identify compounds that stimulate or inhibit the function or level of the polypeptide of any one of claim 1-3 comprising a method selected from the group consisting of:

(a) measuring or, detecting, quantitatively or qualitatively, the binding of a candidate compound to the polypeptide (or to the cells or membranes expressing the polypeptide) or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound;

(b) measuring the competition of binding of a candidate compound to the polypeptide (or to the cells or membranes expressing the polypeptide) or a fusion protein thereof in the presence of a labeled competitior;

(c) testing whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the cells or cell membranes expressing the polypeptide;

(d) mixing a candidate compound with a solution containing a polypeptide of any one of claims 1-3, to form a mixture, measuring activity of the polypeptide in the mixture, and comparing the activity of the mixture to a control mixture which contains no candidate compound; or

(e) detecting the effect of a candidate compound on the production of mRNA encoding said polypeptide or said polypeptide in cells, using for instance, an ELISA assay, and

(f) producing said compound according to biotechnological or chemical standard techniques.

SEQUENCE LISTING

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Gly Lys Met Met Asn Ser His Phe Leu Asp Gly Asn Leu Val Pro Leu
    290                 295                 300

gaa ggg aag gaa gtg gat gag tcc cgc aga gag atg atc cgg att ctc   960
Glu Gly Lys Glu Val Asp Glu Ser Arg Arg Glu Met Ile Arg Ile Leu
305                 310                 315                 320

aag gat ctg aag caa aaa cac cca gag aag gac tta gat cag ctg gtg   1008
Lys Asp Leu Lys Gln Lys His Pro Glu Lys Asp Leu Asp Gln Leu Val
                325                 330                 335

gag atg gcc aat tac tat gct ctt tcc cac caa cag aag agc cgt gcc   1056
Glu Met Ala Asn Tyr Tyr Ala Leu Ser His Gln Gln Lys Ser Arg Ala
            340                 345                 350

ttc tac cgt atc caa gcc act cgt atg atg act ggt gca ggc aat atc   1104
Phe Tyr Arg Ile Gln Ala Thr Arg Met Met Thr Gly Ala Gly Asn Ile
        355                 360                 365

ctg aag aaa cat gca gca gaa caa gcc aag aag gcc tcc agc atg agc   1152
Leu Lys Lys His Ala Ala Glu Gln Ala Lys Lys Ala Ser Ser Met Ser
    370                 375                 380
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gag gtg cac acc gat gag cct gag gac ttt att tcc aag gtc ttc ttt   1200
Glu Val His Thr Asp Glu Pro Glu Asp Phe Ile Ser Lys Val Phe Phe
385                 390                 395                 400

gac cca tgt tct tac cag tgc ctg gag aac tgt ggg gct gta ctc ctg   1248
Asp Pro Cys Ser Tyr Gln Cys Leu Glu Asn Cys Gly Ala Val Leu Leu
                405                 410                 415

aca gtg gtg agg aaa ggg gga gac atg tca aag acc atg tat gtg gac   1296
Thr Val Val Arg Lys Gly Gly Asp Met Ser Lys Thr Met Tyr Val Asp
            420                 425                 430

tac aaa aca gag gat ggt tct gcc aat gca ggg gct gac tat gag ttc   1344
Tyr Lys Thr Glu Asp Gly Ser Ala Asn Ala Gly Ala Asp Tyr Glu Phe
        435                 440                 445

aca gag ggc acg gtg gtt ctg aag cca gga gag acc cag aag gag ttc   1392
Thr Glu Gly Thr Val Val Leu Lys Pro Gly Glu Thr Gln Lys Glu Phe
    450                 455                 460

tcc gtg ggc ata att gat gac gac att ttt gag gag gat gaa cac ttc   1440
Ser Val Gly Ile Ile Asp Asp Asp Ile Phe Glu Glu Asp Glu His Phe
465                 470                 475                 480

ttt gta agg ttg agc aat gtc cgc ata gag gag gag cag cca gag gag   1488
Phe Val Arg Leu Ser Asn Val Arg Ile Glu Glu Glu Gln Pro Glu Glu
                485                 490                 495

ggg atg cct cca gca ata ttc aac agt ctt ccc ttg cct cgg gct gtc   1536
Gly Met Pro Pro Ala Ile Phe Asn Ser Leu Pro Leu Pro Arg Ala Val
            500                 505                 510

cta gcc tcc cct tgt gtg gcc aca gtt acc atc ttg gat gat gac cat   1584
Leu Ala Ser Pro Cys Val Ala Thr Val Thr Ile Leu Asp Asp Asp His
        515                 520                 525

gca ggc atc ttc act ttt gaa tgt gat act att cat gtc agt gag agt   1632
Ala Gly Ile Phe Thr Phe Glu Cys Asp Thr Ile His Val Ser Glu Ser
    530                 535                 540

att ggt gtt atg gag gtc aag gtt ctg cgg aca tca ggt gcc cgg ggt   1680
Ile Gly Val Met Glu Val Lys Val Leu Arg Thr Ser Gly Ala Arg Gly
545                 550                 555                 560

aca gtc atc gtc ccc ttt agg aca gta gaa ggg aca gcc aag ggt ggc   1728
Thr Val Ile Val Pro Phe Arg Thr Val Glu Gly Thr Ala Lys Gly Gly
                565                 570                 575

ggt gag gac ttt gaa gac aca tat ggg gag ttg gaa ttc aag aat gat   1776
Gly Glu Asp Phe Glu Asp Thr Tyr Gly Glu Leu Glu Phe Lys Asn Asp
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gaa act gtg aaa acc ata agg gtt aaa ata gta gat gag gag gaa tac   1824
Glu Thr Val Lys Thr Ile Arg Val Lys Ile Val Asp Glu Glu Glu Tyr
        595                 600                 605

gaa agg caa gag aat ttc ttc att gcc ctt ggt gaa ccg aaa tgg atg   1872
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Glu Arg Gly Ile Ser Gly Val Arg Phe Phe Lys Asp Val Thr Asp Arg
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aag ctg act atg gaa gaa gag gag gcc aag agg ata gca gag atg gga   1968
Lys Leu Thr Met Glu Glu Glu Glu Ala Lys Arg Ile Ala Glu Met Gly
                645                 650                 655

aag cca gta ttg ggt gaa cac ccc aaa cta gaa gtc atc att gaa gag   2016
Lys Pro Val Leu Gly Glu His Pro Lys Leu Glu Val Ile Ile Glu Glu
            660                 665                 670

tcc tat gag ttc aag act acg gtg gac aaa ctg atc aag aag aca aac   2064
Ser Tyr Glu Phe Lys Thr Thr Val Asp Lys Leu Ile Lys Lys Thr Asn
        675                 680                 685

ctg gcc ttg gtt gtg ggg acc cat tcc tgg agg gac cag ttc atg gag   2112
Leu Ala Leu Val Val Gly Thr His Ser Trp Arg Asp Gln Phe Met Glu
    690                 695                 700

gcc atc acc gtc agt gca gca ggg gat gag gat gag gat gaa tcc ggg   2160
Ala Ile Thr Val Ser Ala Ala Gly Asp Glu Asp Glu Asp Glu Ser Gly
705                 710                 715                 720

gag gag agg ctg ccc tcc tgc ttt gac tac gtc atg cac ttc ctg act   2208
Glu Glu Arg Leu Pro Ser Cys Phe Asp Tyr Val Met His Phe Leu Thr
                725                 730                 735

gtc ttc tgg aag gtg ctg ttt gcc tgt gtg ccc ccc aca gag tac tgc   2256
Val Phe Trp Lys Val Leu Phe Ala Cys Val Pro Pro Thr Glu Tyr Cys
            740                 745                 750

cac ggc tgg gcc tgc ttc gcc gtc tcc atc ctc atc att ggc atg ctc   2304
His Gly Trp Ala Cys Phe Ala Val Ser Ile Leu Ile Ile Gly Met Leu
        755                 760                 765

acc gcc atc att ggg gac ctg gcc tcg cac ttc ggc tgc acc att ggt   2352
Thr Ala Ile Ile Gly Asp Leu Ala Ser His Phe Gly Cys Thr Ile Gly
    770                 775                 780

ctc aaa gat tca gtc aca gct gtt gtt ttc gtg gca ttt ggc acc tct   2400
Leu Lys Asp Ser Val Thr Ala Val Val Phe Val Ala Phe Gly Thr Ser
785                 790                 795                 800

gtc cca gat acg ttt gcc agc aaa gct gct gcc ctc cag gat gta tat   2448
Val Pro Asp Thr Phe Ala Ser Lys Ala Ala Ala Leu Gln Asp Val Tyr
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gca gac gcc tcc att ggc aac gtg acg ggc agc aac gcc gtc aat gtc   2496
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            820                 825                 830

ttc ctg ggc atc ggc ctg gcc tgg tcc gtg gcc gcc atc tac tgg gct   2544
Phe Leu Gly Ile Gly Leu Ala Trp Ser Val Ala Ala Ile Tyr Trp Ala
        835                 840                 845

ctg cag gga cag gag ttc cac gtg tcg gcc ggc aca ctg gcc ttc tcc   2592
Leu Gln Gly Gln Glu Phe His Val Ser Ala Gly Thr Leu Ala Phe Ser
    850                 855                 860
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gtc acc ctc ttc acc atc ttt gca ttt gtc tgc atc agc gtg ctc ttg   2640
Val Thr Leu Phe Thr Ile Phe Ala Phe Val Cys Ile Ser Val Leu Leu
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tac cga agg cgg ccg cac ctg gga ggg gag ctt ggt ggc ccc cgt ggc   2688
Tyr Arg Arg Arg Pro His Leu Gly Gly Glu Leu Gly Gly Pro Arg Gly
                885                 890                 895

tgc aag ctc gcc aca aca tgg ctc ttt gtg agc ctg tgg ctc ctc tac   2736
Cys Lys Leu Ala Thr Thr Trp Leu Phe Val Ser Leu Trp Leu Leu Tyr
            900                 905                 910

ata ctc ttt gcc aca cta gag gcc tat tgc tac atc aag ggg ttc       2781
Ile Leu Phe Ala Thr Leu Glu Ala Tyr Cys Tyr Ile Lys Gly Phe
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<213> Homo sapiens

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            20                  25                  30

Gly Gly Ser Gly Asp Val Pro Ser Thr Gly Gln Asn Asn Glu Ser Cys
        35                  40                  45

Ser Gly Ser Ser Asp Cys Lys Glu Gly Val Ile Leu Pro Ile Trp Tyr
    50                  55                  60

Pro Glu Asn Pro Ser Leu Gly Asp Lys Ile Ala Arg Val Ile Val Tyr
65                  70                  75                  80

Phe Val Ala Leu Ile Tyr Met Phe Leu Gly Val Ser Ile Ile Ala Asp
                85                  90                  95

Arg Phe Met Ala Ser Ile Glu Val Ile Thr Ser Gln Glu Arg Glu Val
            100                 105                 110

Thr Ile Lys Lys Pro Asn Gly Glu Thr Ser Thr Thr Thr Ile Arg Val
        115                 120                 125

Trp Asn Glu Thr Val Ser Asn Leu Thr Leu Met Ala Leu Gly Ser Ser
    130                 135                 140

Ala Pro Glu Ile Leu Leu Ser Leu Ile Glu Val Cys Gly His Gly Phe
145                 150                 155                 160

Ile Ala Gly Asp Leu Gly Pro Ser Thr Ile Val Gly Ser Ala Ala Phe
                165                 170                 175

Asn Met Phe Ile Ile Ile Gly Ile Cys Val Tyr Val Ile Pro Asp Gly
            180                 185                 190

Glu Thr Arg Lys Ile Lys His Leu Arg Val Phe Phe Ile Thr Ala Ala
        195                 200                 205
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Trp Ser Ile Phe Ala Tyr Ile Trp Leu Tyr Met Ile Leu Ala Val Phe
210 215 220

Ser Pro Gly Val Val Gln Val Trp Glu Gly Leu Leu Thr Leu Phe Phe
225 230 235 240

Phe Pro Val Cys Val Leu Leu Ala Trp Val Ala Asp Lys Arg Leu Leu
245 250 255

Phe Tyr Lys Tyr Met His Lys Lys Tyr Arg Thr Asp Lys His Arg Gly
260 265 270

Ile Ile Ile Glu Thr Glu Gly Asp His Pro Lys Gly Ile Glu Met Asp
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Gly Lys Met Met Asn Ser His Phe Leu Asp Gly Asn Leu Val Pro Leu
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Glu Gly Lys Glu Val Asp Glu Ser Arg Arg Glu Met Ile Arg Ile Leu
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Lys Asp Leu Lys Gln Lys His Pro Glu Lys Asp Leu Asp Gln Leu Val
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Glu Met Ala Asn Tyr Tyr Ala Leu Ser His Gln Gln Lys Ser Arg Ala
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Phe Tyr Arg Ile Gln Ala Thr Arg Met Met Thr Gly Ala Gly Asn Ile
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Leu Lys Lys His Ala Ala Glu Gln Ala Lys Lys Ala Ser Ser Met Ser
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Glu Val His Thr Asp Glu Pro Glu Asp Phe Ile Ser Lys Val Phe Phe
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Asp Pro Cys Ser Tyr Gln Cys Leu Glu Asn Cys Gly Ala Val Leu Leu
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420 425 430

Tyr Lys Thr Glu Asp Gly Ser Ala Asn Ala Gly Ala Asp Tyr Glu Phe
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Ser Val Gly Ile Ile Asp Asp Asp Ile Phe Glu Glu Asp Glu His Phe
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Ser Tyr Glu Phe Lys Thr Thr Val Asp Lys Leu Ile Lys Lys Thr Asn
675 680 685

Leu Ala Leu Val Val Gly Thr His Ser Trp Arg Asp Gln Phe Met Glu
690 695 700

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Val Phe Trp Lys Val Leu Phe Ala Cys Val Pro Pro Thr Glu Tyr Cys
740 745 750

His Gly Trp Ala Cys Phe Ala Val Ser Ile Leu Ile Ile Gly Met Leu
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Thr Ala Ile Ile Gly Asp Leu Ala Ser His Phe Gly Cys Thr Ile Gly
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Ala Asp Ala Ser Ile Gly Asn Val Thr Gly Ser Asn Ala Val Asn Val
820 825 830

Phe Leu Gly Ile Gly Leu Ala Trp Ser Val Ala Ala Ile Tyr Trp Ala
835 840 845

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850 855 860

Val Thr Leu Phe Thr Ile Phe Ala Phe Val Cys Ile Ser Val Leu Leu
865 870 875 880

Tyr Arg Arg Arg Pro His Leu Gly Gly Glu Leu Gly Gly Pro Arg Gly
885 890 895

Cys Lys Leu Ala Thr Thr Trp Leu Phe Val Ser Leu Trp Leu Leu Tyr
900 905 910

Ile Leu Phe Ala Thr Leu Glu Ala Tyr Cys Tyr Ile Lys Gly Phe
915 920 925

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(71) Applicant (*for all designated States except US*): MERCK PATENT GMBH [DE/DE]; Frankfurter Strasse 250, 64293 Darmstadt (DE).

(72) Inventor; and

(75) Inventor/Applicant (*for US only*): WILM, Claudia [DE/DE]; Dahlienweg 24, 64291 Darmstadt (DE).

(74) Common Representative: MERCK PATENT GMBH; Frankfurter Strasse 250, 64293 Darmstadt (DE).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/83744 A3

(54) Title: NATRIUM-CALCIUM EXCHANGER PROTEIN

(57) Abstract: HNCX3 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing HNCX3 polypeptides and polynucleotides in diagnostic assays.

IN RNATIONAL SEARCH REPORT

International Application No
PCT/EP 01/04886

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/62 C07K14/47 C07K16/18 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NICOLL D A ET AL: "Cloning of a third mammalian Na+-Ca-2+ exchanger, NCX3." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 40, 1996, pages 24914-24921, XP002181612 ISSN: 0021-9258 the whole document --- -/--	1,4,6-8, 10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 October 2001	06. 11. 2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Oderwald, H

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
PCT/EP 01/04886

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KRAEV A ET AL: "The organization of the human gene NCX1 encoding the sodium-calcium exchanger." GENOMICS, vol. 37, no. 1, 1996, pages 105-112, XP002181613 ISSN: 0888-7543 the whole document	1,4,6-8, 10
X	-& DATABASE EMHUM [Online] EMBL Heidelberg, Germany; AC: X91213, 28 January 1998 (1998-01-28) KRAEV A ET AL.: XP002181615 abstract	

X	LI Z ET AL: "Cloning of the NCX2 isoform of the plasma membrane Na+-Ca-2+ exchanger." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 26, 1994, pages 17434-17439, XP002181614 ISSN: 0021-9258 the whole document	1,4,6-8, 10

P,X	WO 00 58473 A (CURAGEN CORP ;LEACH MARTIN (US); SHIMKETS RICHARD A (US)) 5 October 2000 (2000-10-05) see SEQ ID NOs: 2521 and 2522 (pp. 1804-1810) abstract; claims 1-32 page 1 -page 55	1,4-11

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 01/04886

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0058473 A	05-10-2000	AU 3774500 A	16-10-2000
		WO 0058473 A2	05-10-2000

Form PCT/ISA/210 (patent family annex) (July 1992)